Cytoprotective Effect of NC-1300-O-3 against Gastric Lesions Induced by Necrotizing Agents in Rats

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ABSTRACT—The cytoprotective effect of NC-1300-O-3 and its mechanism of action were investigated. NC-1300-O-3 at doses of 3 and 10 mg/kg, p.o. significantly prevented the formation of gastric lesions by HCl·ethanol in rats, and its efficacy was not influenced by repeated administration for up to 4 weeks. The interaction between NC-1300-O-3 and necrotizing agents in the stomach, which is considered to be related to the development of cytoprotection, was not observed. A preventive effect of NC-1300-O-3 against gastric lesions was observed at the same dose even when gastric secretion was completely inhibited by pretreatment with omeprazole. This suggests that the cytoprotective effect of NC-1300-O-3 is an action on the gastric mucosa independent of its antisecretory effect. The cytoprotective effect of NC-1300-O-3 was not affected by pretreatment with indomethacin but was partly decreased by N-ethylmaleimide pretreatment, suggesting the participation of endogenous sulfhydryl compounds in the action of NC-1300-O-3. This compound dose-dependently increased the hexosamine content in the gastric lumen in rats at a dose range of 3–30 mg/kg, p.o. and slightly inhibited a reduction in surface mucus and mucosal hexosamine content caused by necrotizing agents. Moreover, NC-1300-O-3 at doses of 10 and 30 mg/kg, p.o. significantly inhibited the increased gastric vascular permeability caused by alcohol treatment; and at 30 mg/kg, p.o., it inhibited the reduction in potential difference caused by aspirin in rats. These actions were suggested to contribute to the cytoprotective effect of NC-1300-O-3.

Keywords: NC-1300-O-3, Cytoprotective effect, Necrotizing agent, Mucus

It has been reported that proton pump inhibitors such as omeprazole and lansoprazole, with potent antisecretory activity, prevent formation of gastric lesions induced by necrotizing agents such as absolute ethanol (1–4). The cytoprotective effects of these proton pump inhibitors are due to direct actions in the stomach since their cytoprotective effect are clearly observed when administered intragastrically. Omeprazole and lansoprazole are used clinically in the form of enteric coated tablets and granules, respectively, considering that direct cytoprotective effects are hardly reflected in practical ulcer therapy. It has been reported that the new proton pump inhibitor NC-1300-O-3 (2-[[2-(isobutylmethylamino)benzyl]sulfinyl]-1H-benzimidazole), which is being developed as an ordinary tablet formulation, exhibits a potent cytoprotective effect in rats (5) and can inhibit gastric acid secretion even when administered intra-pouch in Heidenhain-pouch dogs (6), suggesting that NC-1300-O-3 has the potential to inhibit gastric acid secretion due to a direct action on the luminal surface. NC-1300-O-3 has also been reported to potently

promote natural healing and delayed healing of acetic acid-induced gastric ulcers in rats (7). NC-1300-O-3 is a unique proton pump inhibitor that exerts both antisecretory and cytoprotective actions and differs from both omeprazole and lansoprazole. In the present study, we elucidated the cytoprotective effect of NC-1300-O-3 and the mechanism of action underlying its cytoprotective effect, using HCl·ethanol, 99.5% ethanol or aspirin as necrotizing agents.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Saitama Experimental Animals Supply, Co., Ltd., Kitakatsushika-gun, Saitama) were housed at a temperature of $22 \pm 2°C$ and a humidity of $55 \pm 10\%$ prior to use. All animals were fasted for 24 hr before the experiments, but were allowed free access to tap water during the fasting period.

HCl·ethanol-induced gastric lesions

Rats (182–264 g) were treated orally with 1 ml of 60% ethanol containing 150 mM HCl (HCl·ethanol) per 200 g body weight (8). One hour after HCl·ethanol treatment, the rats were killed and the stomach removed. The stomach was inflated by injecting 10 ml of 1% formalin to fix the gastric wall. The stomach was subsequently incised along the greater curvature, and the length of each gastric lesion that had developed in the glandular portion was measured under a dissecting microscope (×10) with a square grid. The total length of gastric lesions in each rat was scored as a lesion index. NC-1300-O-3 was given p.o. 30 min prior to HCl·ethanol administration. To study the mechanism of the cytoprotective effect of NC-1300-O-3, indomethacin (5 mg/kg, s.c.; Sigma, St. Louis, MO, USA) and N-ethylmaleimide (NEM, 10 mg/kg, s.c.; Wako, Osaka) or omeprazole (60 mg/kg, i.p.) were injected into each rat at 60 min or 30 min prior to administration of NC-1300-O-3. To preclude a local interaction between HCl·ethanol and NC-1300-O-3, gastric contents were removed from the forestomach under ether anesthesia 30 min after the last dose of a 1-week treatment period in rats (219–287 g), and HCl·ethanol was then administered 5 min later. In rats in which removal of gastric contents was not carried out, only a laparotomy was performed. To study the long-term effect of NC-1300-O-3, rats (181–228 g) were treated for up to 4 weeks. At the time of evaluation of the 1- and 4-week treatment periods, the cytoprotective effect of a single dose of NC-1300-O-3 was also determined using rats that were housed under the same conditions as rats treated repeatedly with NC-1300-O-3.

Determination of the amount of surface mucus on the gastric mucosa

The surface mucus on the gastric mucosa was quantified using the Alcian blue binding method of Kitagawa et al. (9). Briefly, the stomach was removed from the rats and washed with 10 ml of 0.25 mM sucrose. The stomach was infused with 10 ml of 0.1% Alcian blue solution (containing 0.16 M sucrose and 0.05 M CH$_3$COONa and adjusted to pH 5.8 with HCl) for staining. The stomach was kept at room temperature for 2 hr. After removal of the forestomach, the stomach was incised along the lesser curvature, and the glandular portion was everted. The tissue obtained was placed in 10 ml of 0.25 M sucrose for 15 min and then in the same solution for 45 min to remove unbound dye. Bound dye was extracted with 10 ml of 30% dioctyl sodium succinate solution (Tokyo Kasei, Tokyo) in 70% ethanol for 2 hr. After filtration, absorbance of the extract was measured at 620 nm using a spectrophotometer (U-2000; Hitachi, Tokyo). NC-1300-O-3 was administered p.o. to normal rats (197–251 g), and

the stomach was removed 1 hr later. In the rat gastric lesion model (body weight: 251–317 g), NC-1300-O-3 was administered orally 30 min before administration of 99.5% ethanol (1 ml/200 g, p.o.), and the stomach was removed 3 hr later.

Measurement of gastric mucus

Rats (187–252 g) were treated with NC-1300-O-3, p.o. followed by HCl·ethanol (1 ml/200 g, p.o.) 30 min later. One hour after the HCl·ethanol treatment, the stomach was removed and the corpus mucosa was scraped off. Samples from 2 rats were pooled and lyophilized as one sample. Mucus glycoprotein was extracted according to the method of Azuumi et al. (10), and the hexosamine content was determined based on the findings described by Ishihara et al. (11). An aliquot of each lyophilized sample was heated in 50 mM Tris-HCl at 100°C for 3 min and then homogenized. Triton X-100 was added to the homogenate to a final concentration of 2%, and this was then incubated at 37°C for 1 hr to accelerate the extraction of mucin. After centrifugation at 9,000 rpm for 30 min, the supernatant was subjected to hydrolysis in 3 N HCl at 100°C for 4 hr. After neutralization, hexosamine in the hydrolyzed product was assayed according to the method of Neuhaus and Letzring (12) using D-glucosamine as a standard.

For the determination of hexosamine content in the gastric lumen, NC-1300-O-3 was administered orally to rats (202–267 g) and ligation of the pylorus and esophagus was performed 1 hr after drug administration, and the stomach was then removed. According to the method of Chiu et al. (13), the stomach was infused with 2 ml of saline and 1 ml of air and then shaken for 1 min. The gastric content was centrifuged at 3,000 rpm for 15 min, and the supernatant was collected. One milliliter of the supernatant was hydrolyzed with 1 ml of 2 N HCl at 120°C for 16 hr. After neutralization, the hexosamine content was determined by the method of Blix-Gardell (14, 15) using D-glucosamine as a standard. The results were expressed as hexosamine concentration (μg/ml of gastric content) and hexosamine output (μg/rat).

Determination of the glutathione (GSH) level in gastric mucosa

Rats (195–241 g) were treated with NC-1300-O-3, p.o. followed by HCl·ethanol (1 ml/200 g, p.o.) 30 min later. One hour after the HCl·ethanol treatment, the stomach was removed and frozen in liquid nitrogen. The scraped corpus mucosa was weighed and homogenized in 2 ml of phosphate-buffered saline. After deproteinization with 5% trichloroacetic acid and subsequent centrifugation, the supernatant was subjected to GSH determination using the Ellman method (16). The results are expressed as

micromoles per gram wet weight.

Effect on vascular permeability of gastric mucosa

Rats (189–246 g) were orally treated with NC-1300-O-3 or omeprazole. Evans blue (10 mg/kg; Merck, Darmstadt, FRG) was injected into the tail vein 25 min after oral administration of these drugs, and then the rats were treated with 99.5% ethanol (1 ml/200 g) 5 min later. Rats were sacrificed 10 min after ethanol administration, and 2 ml of cold saline was infused into the stomach with the pylorus ligated, and the gastric content was collected. Subsequently, the corpus mucosa was scraped off and its wet weight was measured. According to the method of Katayama et al. (17), the gastric content and mucosa were transferred to a tube containing 3.5 N KOH and incubated overnight at 37°C. A mixture of 4 N H_3PO_4 and acetone (0.7:6.5 v/v) was added, and the resultant solution was centrifuged at 3,000 rpm for 15 min. The absorbance of the supernatant was determined at 620 nm. The amount of dye recovered from the gastric content and the corpus mucosa was expressed as micrograms per milliliter of gastric juice and micrograms per 100 mg tissue, respectively. The total amount of dye was calculated by adding the amount of dye recovered from the gastric content to that recovered from the gastric mucosa and expressed as micrograms per stomach. In another experiment, N-ethylmaleimide (10 mg/kg, s.c.) was administered 30 min prior to NC-1300-O-3 administration, and the same experiment was carried out as described above.

Determination of transmucosal potential difference (PD)

Rats (208–258 g) were anesthetized with urethane (1.2 g/kg, s.c.) and a tracheal cannula was inserted. The stomach was exposed by laparotomy and the cardia was lightly ligated. A polyethylene tube with a three-way cock was inserted into the forestomach from a fistula. A cannula filled with 3% agar-saturated KCl was passed into the stomach through an incision in the duodenum. The cannula was ligated at the pylorus, and the tip of the cannula was positioned in the corpus and used as an intragastric electrode. Another cannula of the same type was inserted into the femoral vein and used as an indifferent electrode. The other ends of the two cannulae were placed in separate beakers filled with saturated KCl solution in which an AgCl electrode was positioned. The potential difference was determined using a pH-mV meter (Model F-7; Horiba, Kyoto) and continuously recorded on a recorder (Rikadenki Kogyo, Tokyo). Each test compound was orally administered during anesthesia. Thirty minutes after administration of test compounds, cannulation of the forestomach and positioning of the electrode were carried out, and the stomach was infused with 3 ml of warmed saline. After 10–15 min, the saline

was replaced by 3 ml of 40 mM aspirin suspension, and the PD was continuously monitored for 1 hr.

Drugs

NC-1300-O-3 and omeprazole were synthesized at the Research Laboratories of Nippon Chemiphar Co., Ltd. and they were suspended in 1% methylcellulose solution. The vehicle and drugs were administered in a volume of 0.5 ml/100 g body weight.

Statistical analyses

All data are expressed as the mean ± S.E. The statistical significance of differences between groups was determined by Dunnett's multiple comparison test. Student's or Aspin-Welch's t-test was also applied to the comparison between two groups. ED_{50} values were calculated from the dose-response relationships using the method of least squares.

RESULTS

Effect of NC-1300-O-3 on HCl·ethanol-induced gastric lesions in rats

In all control rats given the vehicle alone, severe mucosal lesions developed in the glandular stomach 1 hr after HCl·ethanol administration. These lesions consisted of elongated bands of necrosis. Single administration of NC-1300-O-3 at 3 and 10 mg/kg, p.o. reduced the gastric lesion occurrence by 64.2% and 90.0%, respectively. In rats repeatedly given NC-1300-O-3 at 3 and 10 mg/kg, p.o. for 1 week, the inhibition rates were 73.8% and 94.2%, respectively, which were comparable to those induced by a single dose (Fig. 1A). In rats repeatedly administered NC-1300-O-3 at 3 mg/kg, p.o. for 4 weeks, the inhibitory rate (39.7%) was slightly lower than that at 3 mg/kg, p.o. for 1 week, but its effect was the same as that of a single dose (34.3% inhibition at 3 mg/kg, p.o.). In addition, at a dose of 10 mg/kg, p.o., the protective effect (92.4% inhibition) was almost the same as that of a single dose (80.0% inhibition) (Fig. 1B).

Following pretreatment with indomethacin, NC-1300-O-3 at 3 and 10 mg/kg, p.o. significantly prevented gastric lesions induced by HCl·ethanol by 31.2% and 89.2%, respectively, and these results were comparable to those in rats that were not treated with indomethacin. The protective effect of NC-1300-O-3 was barely affected by pretreatment with indomethacin. However, in NEM-treated rats, NC-1300-O-3 had no protective effect at 3 mg/kg, p.o., and the inhibitory rate at 10 mg/kg was 52.1%. The protective effect of NC-1300-O-3 on HCl·ethanol-induced gastric lesions was partially inhibited by NEM pretreatment (Fig. 2).

It was confirmed that gastric secretion was completely
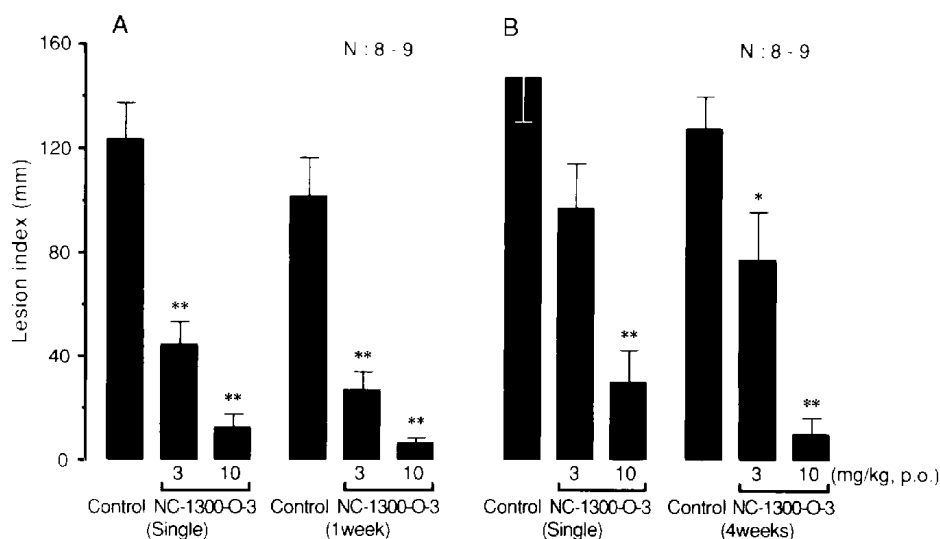
Fig. 1. Effect of NC-1300-O-3 on HCl·ethanol-induced gastric lesions in rats. NC-1300-O-3 was administered p.o. once daily for 4 weeks. HCl·ethanol was given p.o. (1 ml/200 g) 30 min after a single or the last dose of NC-1300-O-3. Animals were sacrificed 1 hr after HCl·ethanol administration. In the case of the single administration groups for 1 week (A) or 4 weeks (B), the rats were kept under the same conditions as rats repeatedly treated with NC-1300-O-3. Each column represents the mean±S.E. *P<0.05, **P<0.01: Significantly different from the control.
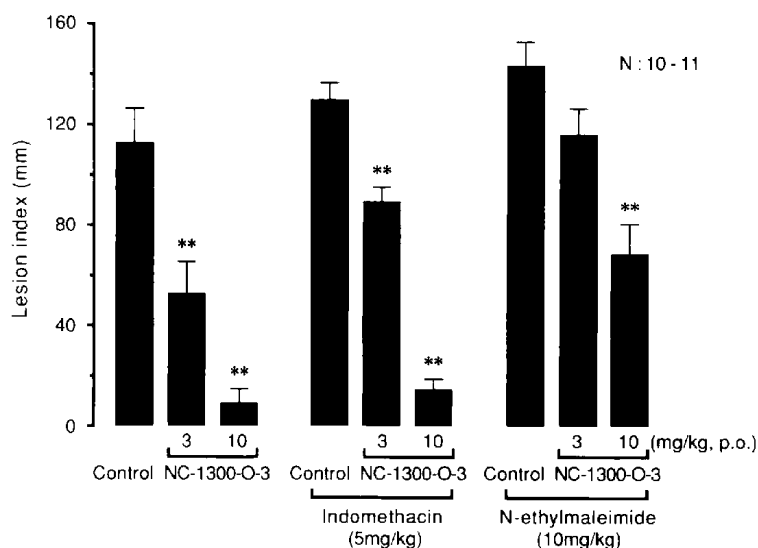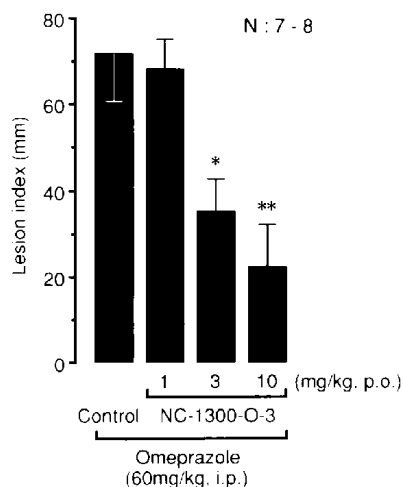

Fig. 2. Effects of indomethacin or N-ethylmaleimide pretreatment on the protective effect of NC-1300-O-3 against HCl·ethanol-induced gastric lesions in rats. Indomethacin (5 mg/kg) or N-ethylmaleimide (10 mg/kg) was administered s.c. 1 hr before NC-1300-O-3 administration. Each column represents the mean±S.E. **P<0.01: Significantly different from the control.

inhibited by omeprazole treatment (60 mg/kg, i.p.) in rats. Omeprazole at 60 mg/kg, i.p. prevented formation of gastric lesions by HCl·ethanol by 34.2% (data not shown). Under such conditions, NC-1300-O-3 also dose-dependently exerted a preventive effect on injury at the same dose as in rats not treated with omeprazole (Fig. 3).

In the experiment where the gastric content was not removed, 1-week successive administration of NC-1300-O-3
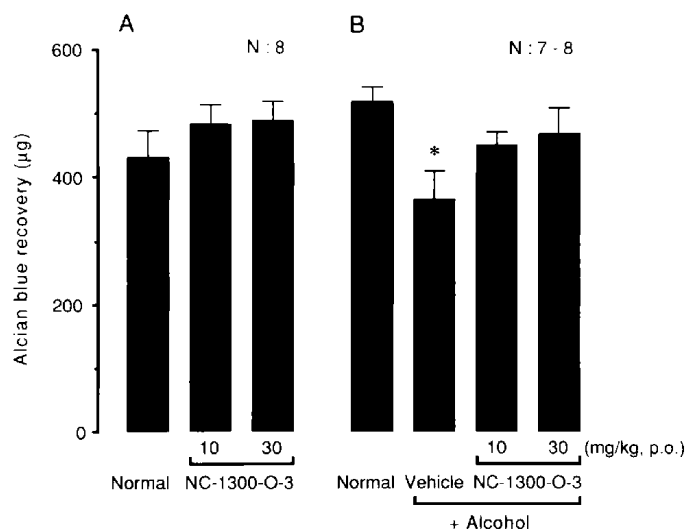
exerted a preventive effect on HCl·ethanol-induced gastric lesions ranging from 23.7% to 98.9% at doses from 3 to 30 mg/kg, and the ED_{50} value was 5.5 mg/kg, p.o. (Fig. 4A). When the gastric content was removed, a similar dose-dependent inhibition ranging from 36.6% to 94.1% was observed at the same dose range, and the ED_{50} value was 4.3 mg/kg, p.o. (Fig. 4B).
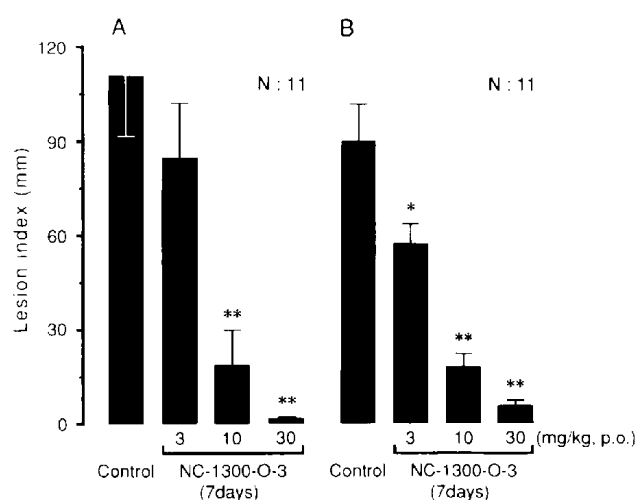
Fig. 3. Effect of omeprazole pretreatment on the protective effect of NC-1300-O-3 against HCl·ethanol-induced gastric lesions in rats. Omeprazole (60 mg/kg) was administered i.p. 30 min before NC-1300-O-3 administration. Each column represents the mean±S.E. *P<0.05, **P<0.01: Significantly different from the control.

Effect on the amount of surface mucus on the gastric mucosa

NC-1300-O-3 at 10 and 30 mg/kg, p.o. slightly increased the amount of mucus 1 hr after administration (Fig. 5A). In rats treated with alcohol, the amount of mucus decreased significantly compared with that in normal rats 3 hr after alcohol administration. NC-1300-O-3 at 10 and 30 mg/kg, p.o. tended to prevent a reduction of the amount of mucus by alcohol treatment (Fig. 5B).

Fig. 4. Effect of NC-1300-O-3 on HCl·ethanol-induced lesions in rats. In (A), NC-1300-O-3 was administered p.o. once daily for 7 days, and HCl·ethanol was administered 30 min after the last dose of NC-1300-O-3. In (B), NC-1300-O-3 was administered p.o. once daily for 7 days and the gastric content of rats was removed 30 min after the last dose of NC-1300-O-3. HCl·ethanol was administered 5 min after removal of the gastric content. Each column represents the mean±S.E. *P<0.05, **P<0.01: Significantly different from the control.

Effect on hexosamine content

NC-1300-O-3 in a dose range from 3 to 30 mg/kg, p.o. increased the hexosamine concentration and output in a dose-dependent manner, and the output at 30 mg/kg was significantly increased (Fig. 6).
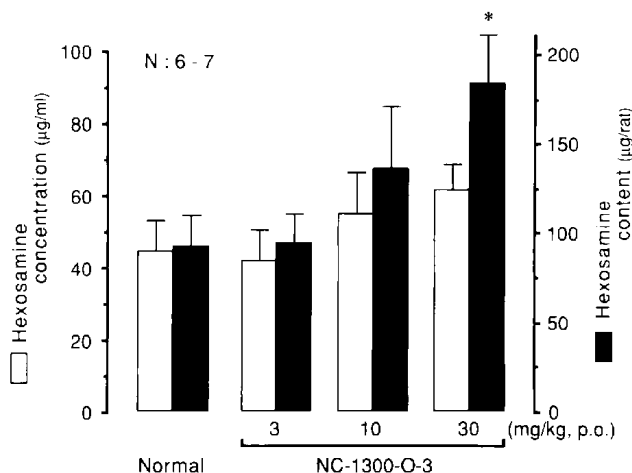

Fig. 5. Effect of NC-1300-O-3 on surface mucus content in gastric mucosa in normal or alcohol-treated rats. In (A), rats were sacrificed 1 hr after oral administration of NC-1300-O-3. In (B), NC-1300-O-3 was administered p.o. 30 min before 99.5% ethanol dosing, and the rats were sacrificed 3 hr later. The amount of Alcian blue bound to the gastric mucosa was determined according to the method described in the text. Each column represents the mean±S.E. *P<0.05: Significantly different from the normal group.

Fig. 6. Effect of NC-1300-O-3 on gastric mucus secretion in rats. NC-1300-O-3 was administered p.o., and the gastric content was collected 1 hr later according to the method described in the text. Each column represents the mean±S.E. *P<0.05: Significantly different from the normal group.

HCl·ethanol-treated rats showed a significantly lower hexosamine content in the gastric mucosa than in normal rats. NC-1300-O-3 tended to prevent a reduction in hexosamine content at 30 mg/kg, p.o. (Fig. 7).

Effect on GSH level in the gastric mucosa

The gastric mucosal GSH level in HCl·ethanol treated rats decreased significantly compared with that in normal

rats. NC-1300-O-3 at doses from 3 to 30 mg/kg, p.o. slightly inhibited the decrease in GSH level, but the effects were independent of dose and the GSH level remained at a significantly lower value than that in normal rats (Fig. 8).

Effect on vascular permeability in gastric mucosa

The amount of Evans blue in the gastric mucosa, gastric content and the total amount significantly increased 10 min after alcohol treatment compared with the values in normal rats, indicating increased vascular permeability in the stomach. NC-1300-O-3 at 10 and 30 mg/kg, p.o. significantly prevented an increase in the amount of Evans blue. In contrast, omeprazole did not prevent the increase in vascular permeability at 30 mg/kg, p.o. (Fig. 9).

It was observed that NEM increased the level of Evans blue in the gastric mucosa compared with the untreated group. Pretreatment with NEM partially inhibited the inhibitory effect of NC-1300-O-3 (30 mg/kg, p.o.) on vascular permeability because NC-1300-O-3 significantly decreased the amount of Evans blue in the gastric mucosa but not in the gastric content or total amount in the NEM-pretreated group (Fig. 10).

Effect on potential difference (PD)

The PD values just before the injection of aspirin in the control and NC-1300-O-3 and omeprazole-treated groups were −31.6±1.5 mV, −30.0±1.7 mV and −40.8±2.8
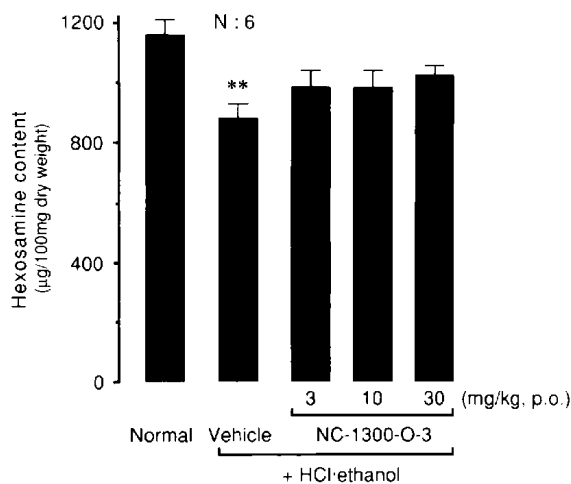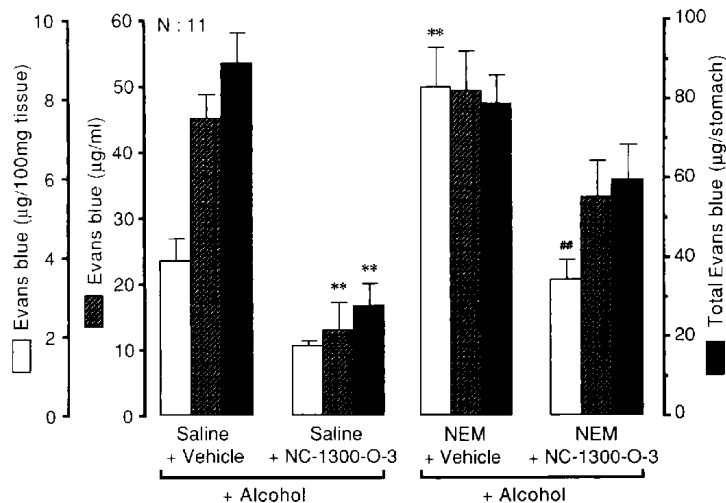

Fig. 7. Effect of NC-1300-O-3 on changes in mucosal hexosamine content caused by HCl·ethanol in rat stomach. NC-1300-O-3 was administered p.o. 30 min before the HCl·ethanol administration, and the rats were sacrificed 1 hr later. The gastric mucosa was collected, and the hexosamine content was determined according to the method described in the text. Each column represents the mean±S.E. **P<0.01: Significantly different from the normal group.
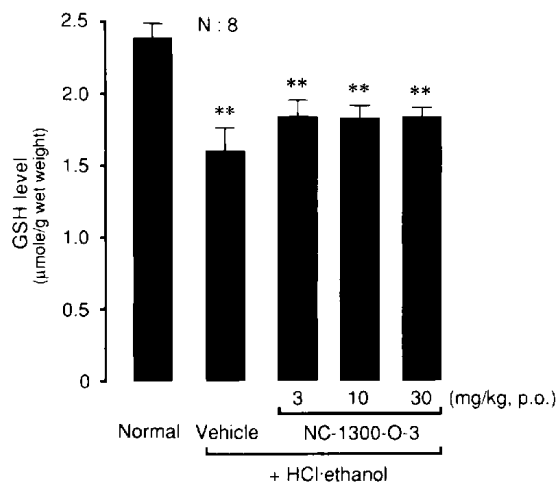
Fig. 8. Effect of NC-1300-O-3 on changes in mucosal glutathione (GSH) level caused by HCl·ethanol in rat stomach. NC-1300-O-3 was administered p.o. 30 min before the HCl·ethanol administration, and the rats were sacrificed 1 hr later. Gastric mucosa was collected and the amount of GSH was determined according to the method described in the text. Each column represents the mean±S.E. **P<0.01: Significantly different from the normal group.
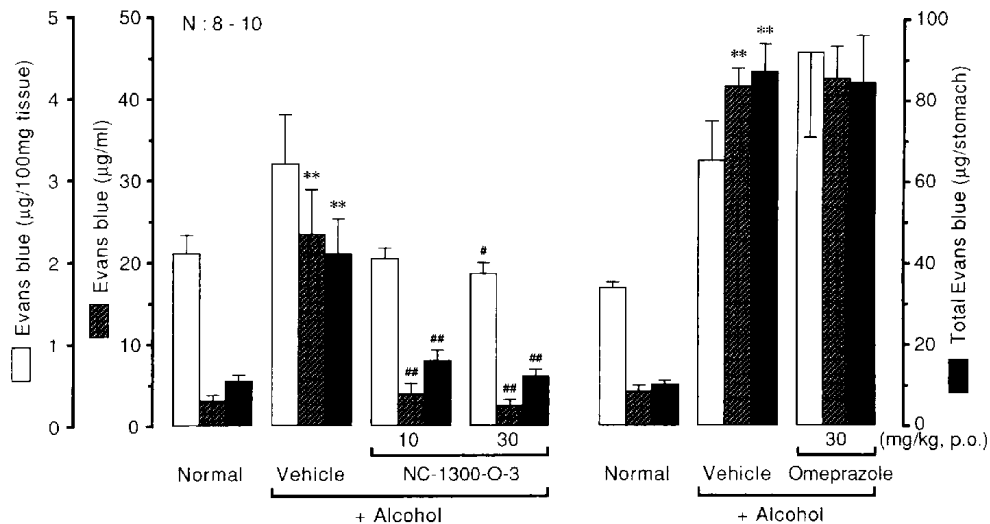
Fig. 9. Effect of NC-1300-O-3 and omeprazole on changes in vascular permeability caused by 99.5% ethanol in the rat stomach. Each drug was administered p.o. 30 min before alcohol administration. Evans blue (10 mg/kg) was administered i.v. 5 min before the alcohol administration, and the rats were sacrificed 10 min later. The amount of Evans blue in the gastric content, gastric mucosa and the total amount were determined according to the method described in the text. Each column represents the mean±S.E. **$P < 0.01$: Significantly different from the normal group, #$P < 0.05$, ##$P < 0.01$: Significantly different from the vehicle+alcohol group.


Fig. 10. Effect of N-ethylmaleimide (NEM) pretreatment on the preventive effect of NC-1300-O-3 on changes in vascular permeability caused by 99.5% ethanol in the rat stomach. NEM was administered s.c. 30 min before NC-1300-O-3 administration. Each column represents the mean±S.E. **$P < 0.01$: Significantly different from the saline+vehicle group. ##$P < 0.01$: Significantly different from the NEM+vehicle group.

mV, respectively. A reduction in PD was observed immediately after the injection of 40 mM aspirin into the stomach, and PD increased gradually from about 20 min after the administration of aspirin. NC-1300-O-3 at 30 mg/kg, p.o. administered about 40 min before aspirin treatment prevented the reduction in PD, and PD rapidly recovered compared to the control. Omeprazole at 30 mg/kg, p.o. showed a comparable effect on PD changes to that caused by NC-1300-O-3 (Fig. 11).

DISCUSSION

It has been confirmed that single oral administration of NC-1300-O-3 at 10 mg/kg or less prevents formation of gastric lesions induced by various necrotizing agents (5), although the cytoprotective effect of repeated administration of this drug has not been clarified. In the present study, the effect of repeated administration of NC-1300-O-3 for up to 4 weeks on gastric lesion formation induced
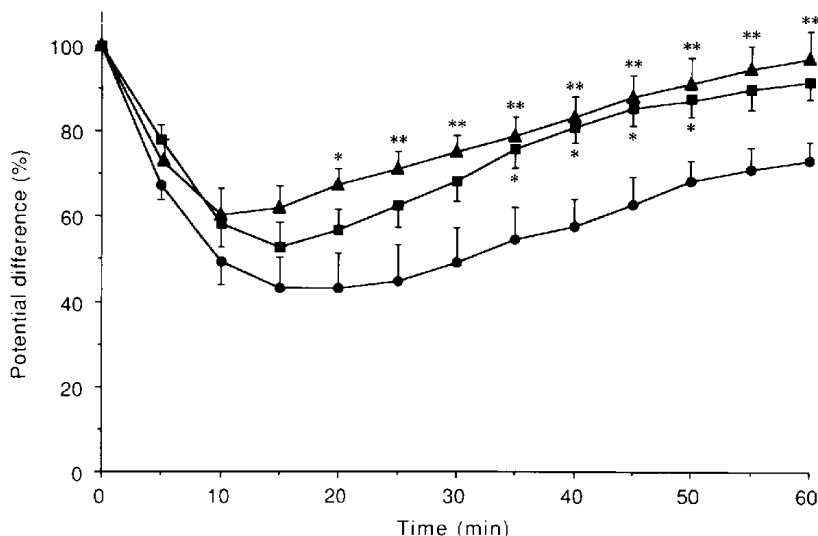
Fig. 11. Effects of NC-1300-O-3 and omeprazole on the changes in potential difference caused by aspirin. Each drug was administered p.o. about 40 min before administration of aspirin (40 mM, 3 ml, i.g.). Each point represents the mean±S.E. *P<0.05, **P<0.01: Significantly different from the control value. ● Control (N=7); ▲ NC-1300-O-3, 30 mg/kg, p.o. (N=8); ■ Omeprazole, 30 mg/kg, p.o. (N=5).

by HCl·ethanol was evaluated. The cytoprotective effect of NC-1300-O-3 observed after repeated administration for 1 week was very similar to that observed following single administration. In the case of repeated administration for 4 weeks, the preventive effect of NC-1300-O-3 at 3 mg/kg was less potent than that seen in 1-week successive dosing, and one reason for this may be that evaluations were carried out in older rats. However, the preventive effect of 4-week successive dosing of NC-1300-O-3 was not different from that following single administration in the same older rats. Consequently, the cytoprotective effect of NC-1300-O-3 is not influenced by successive administration.

The cytoprotective effect of NC-1300-O-3 has been observed to be more potent in the case of oral treatment (5). Therefore, the cytoprotective effect following oral administration of NC-1300-O-3 may contribute to a local interaction between the drug and necrotizing agents in the stomach. In particular, in the presence of increased gastric volume after administration of NC-1300-O-3 (7), physical dilution of the administered necrotizing agents with gastric juice may have a pseudo-effect on the development of gastric lesions. To clarify the effect of such local interaction on the cytoprotective effect of NC-1300-O-3, the preventive effect of NC-1300-O-3 was also evaluated following removal of the gastric content 30 min after the last dose in a 1-week dosing period. The observed effects in this test system were comparable to those observed without removal of the gastric content, excluding the possibility of a pseudo-effect due to physical dilution of or an interaction with necrotizing agents in the stomach.

NC-1300-O-3, administered intragastrically at 100 mg/kg, has been found to have no inhibitory effect on spontaneous gastric motility (18). Takeuchi and Nobuhara (19) have suggested that the increase in spontaneous gastric motility by necrotizing agents is an important factor in the pathogenesis of gastric mucosal lesions caused by such agents and that the cytoprotective action of prostaglandins on gastric lesions is partially related to their inhibitory effect on gastric motility. Therefore, an inhibitory effect on motility is also unlikely to contribute to the cytoprotective effect of NC-1300-O-3 on the gastric mucosa.

It has been considered that the cytoprotective effect of NC-1300-O-3 was independent of its antisecretory effect, since the cytoprotective effect was marked at a dose of less than one third the ED_{50} value on gastric secretion (5). In the present study, NC-1300-O-3 exerted potent antilesion activity on HCl·ethanol-induced gastric lesions under the condition in which gastric secretion was completely inhibited by omeprazole treatment. Consequently, it is suggested that the cytoprotective effect of NC-1300-O-3 is an action on the gastric mucosa independent of its antisecretory effect. Arakawa et al. (20) have suggested that NC-1300 (an analog of NC-1300-O-3) requires gastric acid to exert its cytoprotective action. Therefore, this result also suggests that NC-1300-O-3 does not necessarily require gastric acid to exert its cytoprotective effect, and NC-1300-O-3 itself has a potential cytoprotective effect, although it is unstable in acidic conditions.

Certain cytoprotective compounds have been shown to produce adaptive cytoprotection through stimulation of

prostaglandin biosynthesis in the gastric mucosa (21). We examined whether NC-1300-O-3 acts as a mild irritant mediated by endogenous prostaglandins and found that pretreatment with indomethacin had almost no effect on the protection afforded by NC-1300-O-3. Therefore, it is unlikely that endogenous prostaglandins are involved in the mechanism of the protective action of this drug, and that the effect is different from that of a mild irritant. This is supported by the finding that NC-1300-O-3 administered intragastrically at 30 mg/kg did not reduce the potential difference in rat gastric mucosa (data not shown).

Szabo et al. (22) and Miller et al. (23) have proposed that sulfhydryl compounds in the gastric mucosa play an important role in the cytoprotection seen with several agents. However, controversial data show that sulfhydryl depletors such as diethyl maleate have a cytoprotective effect or that sulfhydryl compounds (mainly reduced glutathione) are not involved in the cytoprotective mechanism (24). We showed that NEM alone had no effect on the formation of HCl·ethanol-induced lesions, and this agent counteracted the cytoprotective effect of NC-1300-O-3 to some extent, thereby suggesting that sulfhydryl compounds may at least partly be involved in the action of NC-1300-O-3. In this study, the effect of NC-1300-O-3 on GSH, a typical endogenous sulfhydryl compound, was evaluated. The level of endogenous GSH showed a marked decrease 1 hr after treatment with HCl·ethanol. This decrease was slightly inhibited by NC-1300-O-3, but the effect was not statistically significant and the decreased GSH level could not be reversed to the normal level in rats. Therefore, it is at least unlikely that NC-1300-O-3 exerts a cytoprotective effect which depends on the GSH level in the gastric mucosa. Dupuy and Szabo (25) carried out comparative biochemical studies on the roles of endogenous sulfhydryl groups using various metals and found that the common factor among the protective agents appears to be the decrease in protein cysteine levels. It is currently unknown if NC-1300-O-3 affects the cysteine levels in gastric mucosa.

Alcohol has been indicated to increase vascular permeability in gastric mucosa and subsequently cause gastric mucosal injury (26). We examined the effect of NC-1300-O-3 on the changes in gastric vascular permeability induced by alcohol. The amount of Evans blue in the gastric mucosa, gastric content and total amount increased in the early phase after administration of alcohol, indicating increased vascular permeability in the stomach. This increased vascular permeability was markedly inhibited by NC-1300-O-3 but not by omeprazole, both at cytoprotective doses. Moreover, the inhibitory effect of NC-1300-O-3 was partially inhibited by pretreatment with NEM. These results suggest that endogenous sulfhydryl

compounds are related to the inhibitory effect of NC-1300-O-3 on the increased vascular permeability induced by alcohol. It is not presently understood why NC-1300-O-3 prevents the increase in vascular permeability induced by alcohol. Since sulfhydryl groups are involved in nonreceptor modulation of the contractile activity of vascular smooth muscle (27), it is considered that the increased vascular permeability caused by NEM may in part be attributed to functional disturbances of such nonreceptor systems responsible for maintenance of normal vascular integrity (28). Consequently, it is speculated that NC-1300-O-3 may act at the same active site as NEM. We have found that NC-1300-O-3 inhibits the luminol-dependent luminescence of neutrophils activated by FMLP or PMA in vitro. It is also thus speculated that NC-1300-O-3 prevents increased vascular permeability due to inhibition of neutrophil-mediated processes, probably by scavenging active oxygen species or by inhibition of myeloperoxidase activity.

Prostaglandins promote gastric mucus secretion as one mechanism of their cytoprotection (29). In normal rats, NC-1300-O-3 at cytoprotective doses slightly increased gastric surface mucus and caused a dose-dependent increase in hexosamine content in the gastric lumen. This agent also slightly inhibited the significant reduction in gastric surface mucus and mucosal hexosamine content caused by necrotizing agents. A weak preventive effect of NC-1300-O-3 against a reduction in mucus caused by necrotizing agents may have resulted from the action of this drug alone on gastric mucus metabolism, since single administration of NC-1300-O-3 to rats was found to elicit secretion of mucin from deep gastric mucosa (K. Ishihara et al., unpublished data). These results demonstrate that NC-1300-O-3 markedly enhances gastric mucus secretion rather than maintaining the gastric mucus under injured conditions, as an effect of the drug on gastric mucus.

In the present study, NC-1300-O-3 inhibited the reduction in potential difference caused by aspirin and facilitated recovery of PD more rapidly than the control. It has been demonstrated that the gastric potential difference decreases following administration of some agents such as necrotizing agents or aspirin and recovers according to the rapid restitution of the injured mucosa (30). Therefore, it was indicated that NC-1300-O-3 enhances the resistance of the gastric mucosa to damage and promotes the restitution of the injured mucosa.

In conclusion, the cytoprotective effect of NC-1300-O-3 is not affected by repeated administration, and its effect is not due to a physical interaction with necrotizing agents in the stomach. Its independent action on gastric mucosa is not due to its antisecretory effect. These results suggest that NC-1300-O-3 exerts a cytoprotective effect by

promoting secretion of gastric mucus, maintaining gastric mucus, enhancing the gastric mucosal resistance and inhibiting increased vascular permeability in the gastric mucosa.

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